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The Effect of Salinity on Root Architecture in Forage Pea (*Pisum sativum* ssp. *arvense* L.)

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ABSTRACT

Background: Plants face different abiotic stresses such as salinity that affect their normal development, growth and survival. Forage pea is an important legume crop for herbage production in ruminants. Its agronomy requires high levels of irrigation and fertilization. This study aimed to evaluate the effect of salinity on seedling root system development in forage pea under semi-hydroponics conditions.

Methods: Different treatment of NaCl doses (0, 50, 100, 150, 200, 250 and 300 mM) on root architecture was investigated in two different forage pea cultivars (Livioletta and Ulubatlı) with contrasting root structures under controlled conditions. The experimental design was completely randomized design with three replications and nine plants per replication.

Result: Salinity affects root and shoot development differently on these cultivars. Despite the salinity, Livioletta produced more shoot (0.71 g) and root biomass (0.30 g) compared to Ulubatlı (0.52 g and 0.25 g for Root and Shoot biomass, respectively) at 150 mM and all other salinity levels. Livioletta developed a better root system and tolerated salt to a higher dose than Ulubatlı. Understanding root system responses of forage pea cultivars may allow breeding and selecting salinity tolerant cultivars with better rooting potential.

Key words: Forage pea, Root architecture, Salinity, Shoot length.

INTRODUCTION

Plants face various abiotic and biotic stressors that affect their development, growth and survival. Abiotic stress factors are environmental limitations such as cold, heat, drought and salinity. Salinity is one of the most important problems limiting plant growth, yield and quality in agriculture (McMaster and Wilhelm, 2003). The area of saline soils in the world continues to increase exponentially. According to estimates, salinity affects about 6% of the arable lands in the globe (FAO, 2018).

Plant roots firstly encounter salinity stress and increased salinity in the soil causes osmotic pressure in the root zone, thus decreasing or preventing the water intake during the germination and the further growth of a plant (Sadeghian and Yavari, 2004). The photosynthesis efficiency, chlorophyll content of leaves (Fedina *et al*. 2003) and transpiration rate and stomatal conductance (Ashraf and Harris, 2004) were also affected by salinity stress. Moreover, salinity can affect nitrogen (Mansour, 2000) and carbon metabolisms (Balibrea *et al.* 2000) and electron transport activity (Sreenivasulu *et al*. 2000). The research demonstrated that the most sensitive growth stage of the plant to salinity is germination and it can significantly reduce and even completely prevent germination (Ahmad *et al.* 2013). Plants respond differently to salinity stress depending on their genetic backgrounds and salinity doses (Almansouri *et al*. 2001; Önal Aşçı and Üney, 2016).

The previous studies showed the effects of salinity in root growth and development in the model and common crops (Talukdar, 2011; Hasan *et al.* 2018; Mann *et al.* 2019). Also, there were several reports of the effect of salinity at the germination stage on forage pea (Demirkol *et al.* 2019; Önal Aşçı and Zambi, 2020), however, no previous reports on the seedling root architecture and salinity interactions were found. During previous studies, significant effects of salinity stress on total root length, root surface area and root thickness in soybean (*Glycine max*) (Egamberdieva *et al.* 2017) and primary root length and root dry weight in *Medicago truncatula* (Ariel *et al*. 2010), were reported. Although forage pea is commonly considered as a moderately salt-tolerant plant species (Berger *et al.* 1999), whereas the salinity-induced response at the seedling stage still needs more exploration (Talukdar, 2011). Considering all these points, the current study aimed to determine the effect of salinity stress on seedling root development of two forage pea cultivars with contrasting root characteristics.

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MATERIALS AND METHODS

Forage pea cultivars Livioletta and Ulubatlı with contrasting rooting characteristics were used in this study (Table 1).

Table 1: The origin, registration date and key characteristics and references of two cultivars evaluated.

Cultivar	Registration date	Origin	Key characteristics	Reference
Livioletta	2018	Maro Tarım İnş. Tic. ve San. A.Ş.	Main stem length: 120.8-167.0 cm Herbage yield: 380.8 kg/ha Hay yield: 81.3 kg/ha Protein ratio: 16.75%	TTSMM, 2018
Ulubatlı	2007	Uludağ University, Faculty of Agriculture	Main stem length: 72.6-81.6 cm Main stem number: 1.3 number/plant Herbage yield: 147.8 kg/ha Hay yield: 37.2-69.3 kg/ha Protein ratio: 13.8-18.8%	Uzun *et al.*, 2012; Seydoşoğlu, 2013

The study was established in a controlled growth chamber in the Department of Agricultural Biotechnology, Siirt University (37°58′13.20″N - 41°50′43.80″E). The temperature and humidity levels were ranged between 25-27°C and 60-70%, respectively. The experiment was conducted under daylight conditions (11.30 h day, 12, 30 h night). The study was established according to completely randomized design (CRD) with three replications and nine plants per replication. A total of six different treatment of NaCl doses (0, 50, 100, 150, 200, 250 and 300 mM) were applied in the study (Demirkol *et al.* 2019).

Seeds were surface sterilized with 70% ethyl alcohol (C_2H_5OH) and 5% sodium hypochlorite (NACIO) for 5 minutes each and rinsed under running water for one minute (Sharma *et al.* 2010). The seeds were imbibed in water for 24 hours for homogeneous germination. Seeds with similar germination were placed between germination papers (40 x 40 cm) as three seeds (10 cm intervals) per germination paper (Zhu *et al.* 2005; Hohn, 2016). The germination papers were rolled and placed in cylindrical containers with a 15 cm water level. The experiment was conducted on October 9th, 2020 and completed on October 24th, 2020.

Root samples were scanned in color scale at 600 DPI with a handheld scanner (Iscan Color Mini Portable Scanner). Root images were analyzed using ImageJ (imagej.nih.gov; Rueden *et al.* 2017) software. The number of roots (NOR), total root length (TRL), average total root length (aTRL), taproot length (TapRL), lateral root length (LatRL), average lateral root length (LatRL/number of lateral roots: aLatRL), shoot length (SL), shoot fresh weight (SFW) and root fresh weight (RFW) were examined (Merrill *et al.* 2002; Ceritoglu *et al.* 2020).

The effect of salinity within and between the cultivars were analyzed with a two-way analysis of variance (ANOVA) using Statistix 10 software (Analytical Software; Tallahassee, FL, USA). Variance groupings were made using Tukey's Honest Significant Difference (HSD) test (Steel *et al.* 1997).

RESULTS AND DISCUSSION

Salinity is a growing threat in agriculture and becoming one of the major limiting factors in crop production. Six different doses of salt (NaCl) were investigated against two different forage pea cultivars with contrasting root characteristics and architectures (Unpublished data). According to variance analysis (ANOVA), salinity caused a significant ($p<0.05$) reduction in most root and growth parameters and shoot length at cultivar, dose and cultivar × dose interactions.

Number of roots

According to our results, the salt application significantly reduced the number of roots (NOR) and the first significant decrease on NOR was seen at 100 mM dose when compared to control (Fig 1). While the first sharpest effect of salinity in Livioletta was observed at 100 mM, for Ulubatlı, a remarkable decrease was observed at 200 mM salinity dose (Fig 1). At the higher doses, both cultivars' NOR values were decreased dramatically. The increase in salinity level changes the osmotic potential. And low osmotic potential reduces the water potential of the soil and therefore water uptake ability of the plant is reduced. As a result, salinity affects many biological events such as growth, development, germination, cell division and photosynthesis (Sadeghian and Yavari, 2004). Previous reports showed that salinity stress reduces root biomass in forage pea (Okçu *et al.* 2005; Önal Aşçı and Eriş, 2019) and our results also showed reduced NOR.

Root and shoot length

The effect of salinity stress on the total root length (TRL) showed that although there were no differences in the root lengths between the control and 50 mM dose, 100 mM salt concentration clearly affected TRL for both cultivars and higher doses impact TRL more dramatically (Fig 2). The TRL varied between 153.2 cm (control) and 5.2 cm (300 mM) (Fig 2). The effects of salinity on the TRL differed for each cultivar. The Livioletta and Ulubatlı did not differ between 0- and 50-mM doses, but Livioletta still had higher TRL values compared to Ulubatlı at the control as well as at all other doses (Fig 2). The level of salinity may vary according to dose, the time elapsed after salt exposure (Hasanuzzaman *et al.* 2013), species (Özkorkmaz and Yılmaz, 2017) and variety (Okçu *et al.* 2005). Egamberdieva *et al.* (2017) reports TRL as 1425.3 cm at 0 mM and 919.2 cm at 75 mM dose in soybean. According to our results, the TRL was decreased as the salt doses increased. Even though low salinity levels may encourage root elongation under mild stress conditions, higher doses generally reduce root growth (Julkowska *et al.* 2014).

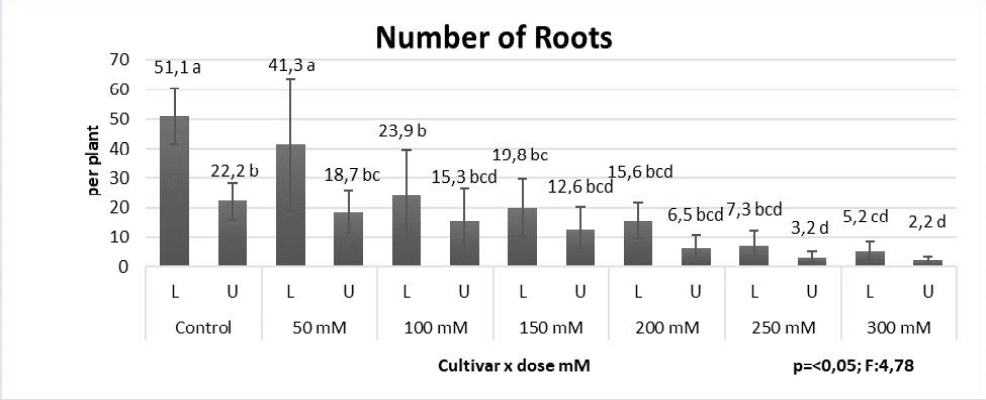


Fig 1: Total number of root values of forage pea cultivars Livioletta (L) and Ulubatlı (U).

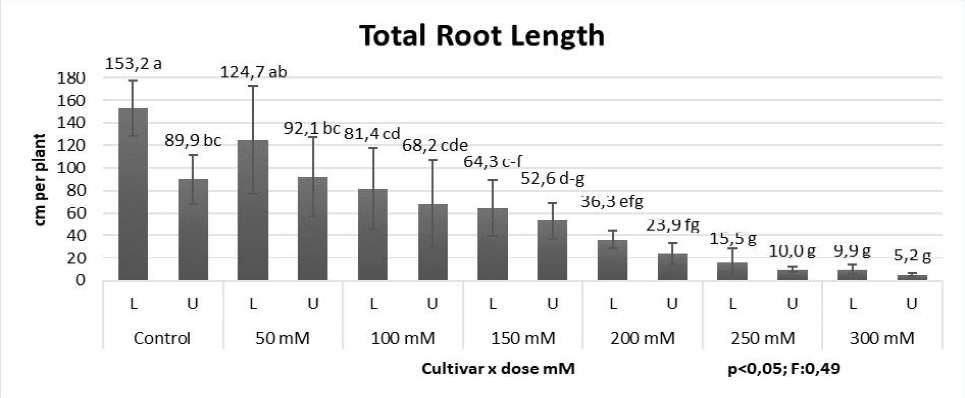


Fig 2: Salt dose × total root length interactions in forage pea cultivars Livioletta (L) and Ulubatlı (U).

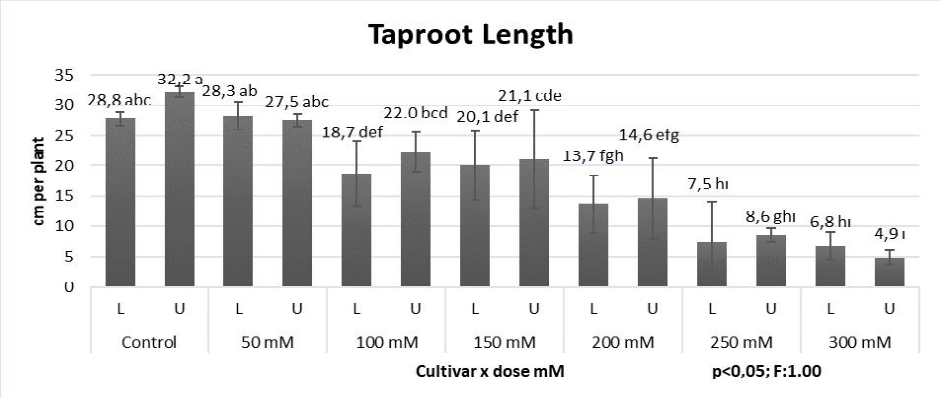


Fig 3: Taproot lengths of forage pea cultivars Livioletta (L) and Ulubatlı (U) under salinity.

According to our results, taproot lengths (TapRL) varied between 32.2 and 4.9 cm (Fig 3). The highest TapRL values were obtained at control and 50 mM doses and from 100 mM concentration, the effect of salinity stress was seen on both cultivars (Fig 3-4). The higher salinity doses, the lower TapRL for both cultivars. Rewald *et al.* (2013) reported that salinity reduces stem elongation by preventing cell expansion and division in the root meristem due to osmotic stress and toxic ions. Besides, high salinity was reported to inhibit the growth of main and lateral roots by suppressing cell division and elongation (Zolla *et al*. 2010). It causes reduced root elongation and limits root surface area, resulting in limited soil coverage and reduced water and nutrient uptake.

Also, average total root length (aTRL) (TRL/NOR) was evaluated to determine the effects of salinity on overall root growth potential. The highest aTRL value was detected in Ulubatlı at 50 mM with 5.08 cm and from 200 mM salt concentration, aTRL values decreased dramatically (Table 2). When the average lateral root length (aLatRL) was examined, the effect of salinity stress began to be seen in Ulubatlı at 200 mM dose. While aLatRL was the highest at 50 mM (3.38 cm), this value decreased to 0.13 cm at 300 mM (Table 2). Lateral root length (LatRL) values were not

significant on cultivar × dose interactions. The reason is thought to be due to higher values at 50 mM compared to control. Although it was not significant, LatRL ranged between 125.4 cm (control) and 0.65 cm (300 mM) for Livioletta (Table 2). No previous studies were reporting the effect of salinity on forage pea seedlings aTRL and aLatRL values, therefore, no direct comparisons were made.

In our results, cultivar × salinity interactions for shoot length (SL) were found ($p<0.05$) significant and each cultivar responded differently to salinity. The highest value was detected in Livioletta with 50.0 cm at 50 mM dose. For Livioletta, there was no significant difference between control and 50 mM and between 100 and 150 mM salinity levels. Livioletta started to be affected at 200 mM, while Ulubatlı

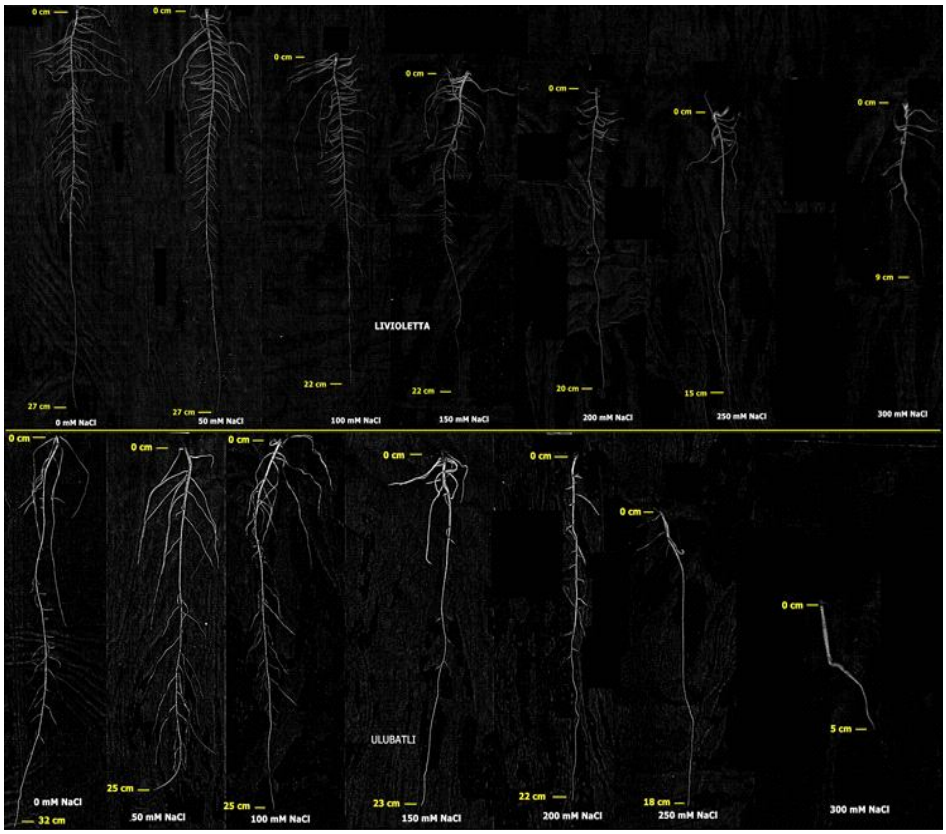


Fig 4: The effect of salinity on root system in forage pea cultivars shown with samples from each dose.

Table 2: Effect of salinity (NaCl) on some root parameters in forage pea.

Salinity	Cultivar	aTRL (cm)	aLatRL (cm)	LatRL (cm)	SL (cm)	RFW (g)	SFW (g)
0 mM	Livioletta	3.11 abc	2.60 abc	125.4	48.7 ab	0.393	0.845 a
	Ulubatlı	4.13 ab	2.72 ab	57.8	31.7 cde	0.357	0.777 ab
50 mM	Livioletta	3.87 abc	2.44 abc	96.5	50.0 a	0.397	0.844 a
	Ulubatlı	5.08 a	3.38 a	64.6	32.5 cd	0.364	0.775 ab
100 mM	Livioletta	3.86 abc	3.04 a	62.7	45.8 abc	0.325	0.768 ab
	Ulubatlı	4.48 ab	3.12 a	48.0	18.7 def	0.324	0.587 a-d
150 mM	Livioletta	2.94 abc	2.08 a-d	44.2	39.6 abc	0.296	0.708 abc
	Ulubatlı	3.58 abc	2.17 a-d	25.7	18.1 def	0.252	0.519 b-e
200 mM	Livioletta	2.52 bc	1.66 a-d	26.9	34.2 bc	0.227	0.510 b-e
	Ulubatlı	2.35 bc	1.14 bcd	16.7	16.5 ef	0.166	0.417 c-f
250 mM	Livioletta	1.64 c	0.94 bcd	8.1	16.1 f	0.136	0.285 def
	Ulubatlı	2.28 bc	0.43 d	1.8	8.6 f	0.097	0.178 f
300 mM	Livioletta	1.26 c	0.65 cd	3.0	14.1 f	0.086	0.216 ef
	Ulubatlı	1.07 c	0.13 d	0.6	4.1 f	0.067	0.081 f
F value		0.49*	0.94*	3.99	2.04*	0.28	0.29*

TRL: Average total root length; aLatRL: Average lateral root length; LatRL: Lateral root length; RFW: Root fresh weight; SFW: Shoot fresh weight; *:$p< 0.05$.

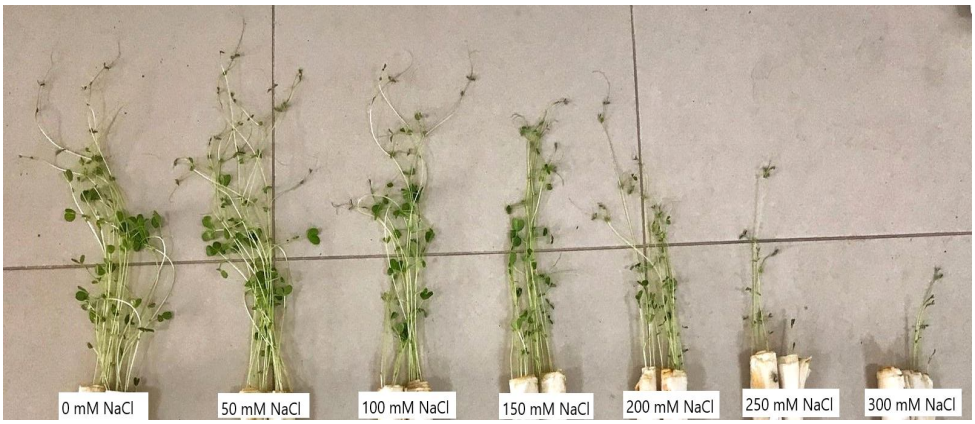


Fig 5: Shoot development under salinity (0-300 mM).

was affected at 100 mM dose (Table 2). Different responses of cultivars within a species against increased salinity may be due to higher photosynthesis potential and as a result, shoot and root development in Livioletta. McPhee (2005) reports 5.9 to 38.0 cm SL in pea accessions, Önal Aşçı and Eriş (2019) reports SL between 37.52 and 42.67 cm, Önal Aşçı and Zambi (2020) reports high SL values at 50 mM salt dose, while significant decreases were observed as the salt dose increased in forage pea. The results obtained in this study were similar to previous reports. Salinity may enhance shoot growth up to a certain dose and seems to limit growth after that threshold.

Root and shoot biomass

The effect of cultivar × salinity interactions on shoot fresh (SFW) weights was found to be statistically significant ($p<0.01$). The highest SFW values were found in Livioletta at control (0.845 g) and 50 mM (0.844 g) doses. The sharpest effect of salinity on SFW was observed at 150 mM in Ulubatlı, while the same effects were seen at 200 mM dose in Livioletta. For Ulubatlı, the lowest values were 0.178 and 0.081 g in 250- and 300-mM doses, respectively (Table 2, Fig 5). Demirkol *et al.* (2019) reported reduced SFW at 30 mM, while Maksimovic *et al.* (2010) reported decreased water content in the leaves and stems in pea and this caused a reduction in SFW. Önal Aşçı and Zambi (2020) reported that 25 mM salt dose started to reduce SFW in forage pea. In the current experiment, a higher salinity tolerance compared to previous reports were observed. This may be due to differences in cultivars or experimental systems.

CONCLUSION

According to our results, the major growth-limiting threshold of salinity on forage pea was 100 mM dose. However, each cultivars' threshold was different, Livioletta had a higher salt tolerance, compared to Ulubatlı. After a certain dose (200 mM), cultivar differences were not observed, meaning too high of a dose for both. Screening salinity tolerance levels of cultivars and germplasm accessions under controlled and field conditions may allow the identification of novel alleles to develop salinity tolerant cultivars. There is a need for the evaluation of neglected crops, such as forage pea, for salinity tolerance under high-throughput conditions to better understand genotypic responses.

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